RELATIONSHIP OF ALKALI INSOLUBLE COLLAGEN TO TENDERNESS OF THREE BOVINE MUSCLES

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CONNECTIVE tissue collagen has been implicated for many years as contributing to the tenderness of meat. Husaini *et al.* (1950) showed a significant negative correlation between collagen content and tenderness in beef at 15 days post-mortem. Wierbicki *et al.* (1955) found that bulls tended to be less tender and to have a higher collagen content than steers. Other workers, however, have found little or no relationship between collagen content and meat tenderness. Herschberger *et al.* (1951) observed no significant correlation between collagen nitrogen content and beef tenderness, and Carpenter *et al.* (1963) found no relationship between total collagen content and muscle tenderness. Ritchey *et al.* (1963) reported that residual collagen remaining after cooking had a definite effect on tenderness. Irwin and Cover (1959) reported collagen nitrogen to be considerably lower in cooked *longissimus dorsi* muscle than in *biceps femoris,* and tenderness followed the same trend. However, Skelton *et al.* (1963) observed higher collagen nitrogen values for cooked meat than for uncooked meat.

This investigation was undertaken to study the relationship of alkali-insoluble collagen to shear value of bovine muscles in an effort to clarify the role and specific relationship of collagen in the tenderness of beef muscle.

Materials and Methods

One hundred eighteen yearling steer carcasses were surveyed initially for tenderness using shear values of the *l. dorsi* from the 12th rib taken 7 days post-partum. From this group 28 carcasses were selected, based upon preliminary shear values, and grouped according to shear values into tender (less than 11.3 kg. shear on a 2.5-cm. core) and less tender (more than 13.6 kg. shear on a 2.5-cm. core) groups. Nine days after slaughter steaks 2.5-cm. thick were taken from the *l. dorsi, semimembranosus* and *triceps brachii* muscles. These steaks were cooked in deep fat (135° C.) to an internal temperature of 70° C., and three 2.5-cm. cores were removed and sheared on the Warner-Bratzler shearing device. These shear values were used in relating tenderness to alkali-insoluble collagen. Samples were frozen and stored at 0° C. for subsequent chemical analysis.

Alkali-insoluble Collagen Extraction Procedures. Alkali-insoluble collagen was determined in both the uncooked and cooked samples, utilizing a modification of the extraction procedure described by Ritchey *et al.* (1963). Samples were ground fresh, placed in glass jars, tightly sealed, frozen and stored at 0° C. until used. A 5-gm. sample thawed at 5° C. for 24 hr. was used to determine total collagen nitrogen in the uncooked meat, and a 2.5-gm. sample was used for cooked meat. The samples were weighed into 60-ml. polyethylene centrifuge tubes, 40 ml. of distilled water was added, and the tubes were placed on a shaker at high speed for 30 min. Tubes were then centrifuged for 10 min. at 4,000 rpm and the supernatant was passed through a coarse-sintered glass filter. Forty milliliters of water was added to the sample, and 5 ml. of 1.0 N sodium hydroxide was slowly added while the mixture was stirred. Stirring was continued for 2 hr., and the centrifugation and filtration steps were repeated. Forty milliliters of 0.1 N sodium hydroxide was then added, and stirring was repeated for 2 hr. Samples remained in sodium hydroxide overnight during the second alkaline extraction, and were then filtered through a coarse-sintered glass filter with a glass wool pad. Cooked meat samples were washed with warm water (45 to 48° C.) to remove gelatin, transferred to Erlenmeyer flasks, and diluted with approximately 20 ml. of water. The

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flasks were then capped with beakers. Samples were autoclaved for 6 hr. at 120° C., and filtered while hot through medium-sintered glass filters. The residue was washed several times with boiling water, filtered and added to the filtrate. The filtrate was transferred to a 50-ml. volumetric flask and brought to volume. Ten milliliters of filtrate and 10 ml. of concentrated hydrochloric acid were sealed in a glass ampule and autoclaved at 120° C. for 15 hr. Contents of the ampule were neutralized using 0.1 N sodium hydroxide and brought to a final volume of 250 ml. for hydroxyproline analysis.

Hydroxyproline Determination. Hydroxyproline was measured on an aliquot of the alkali-insoluble, autoclave-soluble filtrate by a modification of the procedure of Woessner (1961) as described by McClain *et al.* (1965).

Collagen calculation from hydroxyproline content was made using 13% as the conversion figure (Gross *et al.,* 1954). Collagen nitrogen content of the samples was calculated, assuming 18.6% nitrogen in collagen (Ritchey *et al.,* 1963), and expressed as percent of total nitrogen in the sample.

Results

Shear Values. Mean shear values for the three muscles used in this study are shown in table 1. Analysis of variance revealed a significant ($P<.01$) difference between shear values of *l. dorsi* muscles in the tender and less tender groups (table 2). No significant differences were observed in shear values of *semimembranosus* muscles. Shear values differed ($P<.05$) by 1.3 kg. of shear force in *triceps brachii* muscles between the tenderness groups. *L. dorsi* muscles were significantly ($P<.01$) different from the *semimembranosus* or *triceps brachii* muscles in the less tender group (table 3). These findings substantiated work by Cover *et al.* (1962) showing that variation in shear force in one muscle accounted for less than 36% of the variation in other carcass muscles.

Alkali-insoluble Collagen in Uncooked Meat. No significant differences in the quantity of alkali-insoluble collagen were found between tenderness groups for *l. dorsi* or *triceps brachii* muscles, and only a slight ($P<.05$) difference was observed in the *semimembranosus* muscles (table 2). However, there was a significant ($P<.01$) difference in alkali-insoluble collagen content between muscles within tenderness groups (table 3). The less tender *l. dorsi* muscles averaged 15 kg. in shear force and contained 2.2% alkali-insoluble collagen, while the tender *l. dorsi* muscles averaged 8.3 kg. in shear force and had 2.5% alkali-insoluble collagen (table 1). Little difference in shear values was observed in the other muscles; yet large differences were found in the content of alkali-insoluble collagen.

Alkali-insoluble Collagen in Cooked Meat. The quantity of residual collagen in muscles after cooking is shown in table 1. Analysis of variance revealed no significant differences in collagen content of cooked meat among muscles studied or between tenderness groups. However, regardless of initial collagen content, muscles tended to reach a relatively constant collagen content upon cooking. The *l. dorsi* muscles, with an average shear value of 15.0 kg., contained 0.51% collagen when cooked while the *semimembranosus* muscles of the same group had an average shear value of 15.0 kg. and contained 0.67% alkali-insoluble collagen. These differences in quantity of alkali-insoluble collagen were small and nonsignificant and appeared to indicate a diminished role for the quantity of alkali-insoluble collagen in tenderness of cooked meat.

The *l. dorsi* muscles of the less tender group contained an average of 2.2% alkali-insoluble collagen before cooking and 0.51% after cooking, while *semimembranosus* muscles averaged

TABLE 1. MEAN SHEAR VALUES AND ALKALI-INSOLUBLE COLLAGEN IN COOKED AND UNCOOKED BEEF MUSCLE

		Longissimus dorsi			*Semimembranosus*			*Triceps brachii*		
			Alkali-insoluble collagen [a]			Alkali-insoluble collagen [a]			Alkali-insoluble collagen [a]	
Tenderness group	N	Shear value [b]	Uncooked	Cooked	Shear value [b]	Uncooked	Cooked	Shear value [b]	Uncooked	Cooked
Less tender	14	15.00	2.20	0.51	9.18	3.14	0.67 [c]	9.64	4.98	0.97 [c]
S. E.		0.97	0.31	0.18	0.42	0.12	0.09	0.39	0.90	0.23
Tender	14	8.37	2.50	0.28	8.48	3.65	0.56 [d]	8.34	5.20	0.80 [d]
S. E.		0.49	0.25	0.07	0.36	0.21	0.12	0.35	0.39	0.20

[a] Collagen protein expressed as a percent of total protein.
[b] Kilograms of shear force on a 2.5-cm. core.
[c] N=7.
[d] N=6.

TABLE 2. ANALYSIS OF VARIANCE FOR SHEAR VALUE AND ALKALI-INSOLUBLE COLLAGEN BETWEEN TENDERNESS GROUPS IN THREE MUSCLES

Source of variation	d.f.	Mean squares: Shear value	Alkali-insoluble collagen: Uncooked	Alkali-insoluble collagen: Cooked
Longissimus dorsi				
Total	27			
Treatment	1	308.29**	0.60	0.37
Error	26	8.30	0.50	0.12
Semimembranosus				
Total	27			
Treatment	1	3.41	1.78*	0.04
Error	26	2.12	0.40	0.08[a]
Triceps brachii				
Total	27			
Treatment	1	11.86*	0.36	0.09
Error	26	1.90	1.63	0.30[a]

* P<.05.
** P<.01.
[a] d.f.=11.

TABLE 3. ANALYSIS OF VARIANCE FOR SHEAR VALUES AND ALKALI-INSOLUBLE COLLAGEN BETWEEN MUSCLES IN TWO TENDERNESS GROUPS

Source of variation	d.f.	Mean squares: Shear value	Alkali-insoluble collagen, uncooked
Tender group			
Total	41		
Muscles	2	0.07	25.72**
LD vs. SM & TB[a]	1	0.02	34.43**
SM vs. TB	1	0.13	17.00**
Error	39	2.26	1.20
Less tender group			
Total	41		
Muscles	2	146.73**	27.70**
LD vs. SM & TB	1	291.98**	31.98**
SM vs. TB	1	1.54	23.68**
Error	39	5.95	0 49

[a] LD=*longissimus dorsi*, SM=*semimembranosus* and TB=*triceps brachii*.
* P<.05.
** P<.01.

3.14 and 0.67% in the uncooked and cooked steak, respectively. Uncooked *triceps brachii* muscles averaged 4.98% alkali-insoluble collagen and 0.97% when cooked. These values represented a loss of collagen during cooking of 67, 79 and 81%, respectively, for the three muscles. The differences in percent loss of collagen were significant (P<.05), which indicated there were differences in types of characteristics of the collagen present.

Discussion

Researchers have devoted many years to search for a definition of the elusive quality characteristic of meat called "tenderness". Much knowledge has been gained, but the exact nature of tenderness and the factors affecting it are still a matter for speculation.

Shear value data have indicated that the *l. dorsi* muscle was subject to the greatest variation in tenderness. Among the three muscles studied, the *l. dorsi* was the only muscle which yielded high shear values.

Results of this study indicated that absolute quantities of alkali-insoluble collagen were not related to shear values in uncooked or cooked muscles. However, differences were observed in the percent alkali-insoluble collagen converted to gelatin upon cooking, indicating differences in types or characteristics of the muscle collagen. Goll *et al.* (1964) reported that maturation of collagen was accompanied by the formulation of stronger crosslinkages within the tropocollagen molecule, and that increases in thermal shrinkage temperature would be expected in older animals. This conceivably could alter the amount of collagen converted to gelatin during cooking, which ultimately would affect meat tenderness. Absolute quantities of connective tissue collagen may not be the most important criteria. The physical and chemical state of the connective tissue may affect tenderness of muscle tissue. This theory was substantiated by Wilson (1959), who reported the sarcolemma lost some of its semipermeable characteristics during onset of rigor mortis, thus permitting a greater interchange of electrolytes. This in turn could greatly affect the ionic atmosphere and water binding characteristics and subsequently the tenderness of muscle tissue.

There has been general agreement that collagen fibers, when first laid down, conform in all respects to the histological definition of reticulin. Bloom and Fawcett (1962) stated that reticular fibers were continuous with collagenous fibers, and that there was a gradual transition of one into the other. Some workers have considered reticular fibers to be immature collagenous fibers and called them "precollagenous" fibers. Gross (1961) theorized that the anabolism of collagen occurred through the conversion of salt-soluble collagen to acid-soluble collagen to mature collagen. McClain *et al.* (1965) reported the presence of acid- and salt-soluble fractions of collagen which were not measured by conventional methods for collagen analysis, and could result in an underestimation of the true collagen content of muscle tissue. These soluble fractions were found to be altered by age of animal and post-mortem aging, and could conceivably play a role in meat tenderness. Although the data indicate that absolute quantities of alkali-insoluble collagen do not appear to be related to muscle tenderness, the possibility should not be overlooked that certain connective tissue constituents, although minimal in quantity, may well play a determining role in the architectural aspects and, subsequently, the tenderness of meat.

Summary

The *l. dorsi, semimembranosus* and *triceps brachii* muscles from the carcasses of 28 yearling steers, classified as tender or less tender on the basis of shear values of the *l. dorsi* muscles were utilized in a study of the specific relationship of alkali-insoluble collagen to meat tenderness. The *l. dorsi* was the only muscle studied which varied greatly in tenderness. Alkali-insoluble collagen in raw meat was found to differ significantly among muscles, but did not differ significantly between tenderness groups. The quantity of alkali-insoluble collagen remaining after cooking was relatively constant among muscles.

Literature Cited

Bloom, William and Don W. Fawcett. 1962. A Textbook of Histology (8th ed.). W. B. Saunders Company, Philadelphia, Pa.

Carpenter, Z. L., R. G. Kauffman, R. W. Bray, E. J. Briskey and K. G. Weckel. 1963. Factors influencing quality of pork. A. Histological observations. J. Food Sci. 28:467.

Cover, Sylvia, Robert L. Hostetler and S. J. Ritchey. 1962. Tenderness of beef. IV. Relations of shear force and fiber extensibility to juiciness and six components of tenderness. J. Food Sci. 27:527.

Goll, D. E., W. G. Hoekstra and R. W. Bray. 1964. Age associated changes in bovine muscle connective tissue. II. Exposure to increasing temperature. J. Food Sci. 29:615.

Gross, J. 1961. Collagen. Scientific American 204(5): 88.

Gross, J., J. H. Highberger and F. O. Schmitt. 1954. Extraction of collagen from connective tissue by neutral salt solutions. Proc. Nat. Acad. Sci. 41:1.

Herschberger, T., R. Deans, L. E. Kunkle, P. Gerlaugh and F. E. Deatherage. 1951. Studies on meat. III. The biochemistry and quality of meat in relation to certain feeding management practices. Food Technol. 5:523.

Husaini, S. A., F. E. Deatherage, L. E. Kunkle and H. N. Draudt. 1950. Studies on meat. I. The biochemistry of beef as related to tenderness. Food Technol. 4:313.

Irwin, L. and Sylvia Cover. 1959. Effect of a dry heat method of cooking on the collagen content of two beef muscles. Food Technol. 13:655.

McClain, P. E., A. M. Mullins, S. L. Hansard, J. D. Fox and R. F. Boulware. 1965. Acid and salt soluble collagen in bovine muscle. Proc. Soc. Exp. Biol. Med. 119:492.

Ritchey, S. J., Sylvia Cover and R. L. Hostetler. 1963. Collagen content and its relation to tenderness of connective tissue in two beef muscles. Food Technol. 17:76.

Skelton, M., D. L. Harrison, S. L. Hall, R. F. Adams and G. E. Goertz. 1963. Effect of degree of doneness on collagen nitrogen and collagenous tissue in two muscles of beef. Food Technol. 17:93.

Wierbicki, E., V. R. Cahill, L. E. Kunkle, E. W. Klosterman and F. E. Deatherage. 1955. Effect of castration on biochemistry and quality of beef. J. Agr. Food Chem. 3:244.

Wilson, G. D. 1959. Physical characteristics of muscle tissues as related to imbibed water. Proc. Recip. Meat. Conf. 12:97.

Woessner, J. F., Jr. 1961. The determination of hydroxyproline in tissue and protein samples containing small proportions of this amino acid. Arch. Biochem. Biophy. 93:440.